Ecological aspects of influenza A viruses in animals and their relationship to human influenza: a review[1]

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It is a reflection of the continuing importance of influenza that it has been termed 'the last great plague of man' (Kaplan & Webster 1977, Beveridge 1977). In many respects influenza in man is unique in its epidemiology, most particularly in that at intervals as short as 10 years or as long as 40 a 'new' virus emerges which results in worldwide infections of a large proportion of the human population (Pereira 1980). Such pandemics may have an enormous impact on society in terms of mortality, morbidity and economic factors. Because of these effects there are detailed accounts of recognizable influenza pandemics which date back many centuries. Possibly the most devastating influenza pandemic recorded occurred in 1918. It has been estimated that during this pandemic between 20 and 40 million deaths occurred throughout the world and that in a developed country, such as the USA, about 0.5% of the population died (Kaplan & Webster 1977).

Aetiology

The influenza viruses form the Orthomyxoviridae family, which has one genus consisting of type A and type B viruses. Type C viruses represent a probable second genus but this has not yet been ratified (Matthews 1979). All three types of influenza virus infect man, but, except for occasional reports, infections of other animals are restricted to type A influenza viruses. Only influenza A viruses have been isolated from birds. Type A and B viruses both cause similar clinical disease in man and both may be responsible for epidemics in man. However only influenza A viruses have produced the devastating pandemics that have made such an impact on the human population throughout recorded history.

Influenza A virus particles appear roughly spherical or filamentous, 80–120 nm in diameter or cross section, by negative contrast electron microscopy. The nucleocapsid shows helical symmetry and is enclosed within a protein matrix. External to the matrix is a lipid membrane, the surface of which is covered by two types of glycoprotein projections, or spikes, with which haemagglutinin and neuraminidase activities are associated. These two surface glycopolypeptides, particularly the haemagglutinin, appear to be the most important antigens in terms of the stimulation of protective immunity in the host. Consequently, considerable antigenic variation is seen in these polypeptides while other polypeptides are antigenically more static.

The classification and nomenclature of influenza viruses take account of the antigenic variation that exists. The rules used are based on the recommendations of the World Health Organization (WHO) Expert Committee (1971), which have been more recently revised (WHO Expert Committee 1979, 1980). Influenza viruses are grouped into types A, B or C on the basis of the antigenic nature of the internal nucleocapsid or the matrix protein. Both these antigens are common to all viruses of the same type (Schild 1972). Viruses of influenza A type are further divided into subtypes on the basis of the haemagglutinin (H) and neuraminidase (N) antigens. The nomenclature recommended by the WHO Expert Committee in 1971 involved the use of a suffix to the H or N subtype (but not for virus of human origin) to denote the animal from which the virus was isolated, e.g. Hsw1, Heq2, Hav4. However, it became clear that no such host range specificity actually existed and the revised system recommended in 1980 dispensed with these suffixes. In addition, more sophisticated techniques had by then

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Table 1. Subtypes of influenza A viruses

Haemagglutinin subtypes		Neuraminidase subtypes	
Current	Previous	Current	Previous
H1	H0, H1, Hsw1	N1	N1
H2	H2	N2	N2
H3	H3, Heq2, Hav7	N3	Nav2, Nav3
H4	Hav4	N4	Nav4
H5	Hav5	N5	Nav5
H6	Hav6	N6	Nav1
H7	Heq1, Hav1	N7	Neq1
H8	Hav8	N8	Neq2
H9	Hav9	N9	Nav6
H10	Hav2		
H11	Hav3		
H12	Hav10		
H13●			

●First isolation made after introduction of current nomenclature
Current: WHO Expert Committee 1980
Previous: WHO Expert Committee 1971

become available for determining antigen similarity (Schild *et al.* 1980, Scholtissek 1978) which revealed close relationships between subtypes originally thought to be distinct. The old and current systems of nomenclature for influenza A subtypes are shown in Table 1. There are, at present, 13 H and 9 N subtypes; a virus possesses one H and one N subtype, apparently in any combination (Hinshaw *et al.* 1981*b*).

In influenza A viruses the genome is single-stranded RNA which is transcribed to complementary messenger RNA by a virus-associated transcriptase. The RNA is present as eight discrete segments which can be regarded as monocistronic in that each segment codes for one polypeptide (this is a simplification; *see* Inglis & Almond 1980). Because the RNA is segmented, genetic reassortment (sometimes termed recombination) can readily take place in mixed infections with different strains of influenza A viruses (Webster *et al.* 1973, Webster & Laver 1975). This means that when two viruses infect the same cell, progeny viruses may contain sets of RNA segments made up of combinations of segments identical to those from either of the parent viruses. This gives a possible number of 2^8 (=256) different combinations that can form a complete set of RNA segments in theoretically viable progeny from a dual infection. In terms of surface antigens this would mean that if, for example, a dual infection occurs with viruses of H1N1 and H3N2 antigens, then the progeny may possess H1N1, H1N2, H3N2 or H3N1 combinations of antigenic subtypes. However, any combination of the other six RNA segments may also occur, so that progeny virus of H3N2 antigenic subtype may possess up to six other segments (or genes) from the H1N1 parent virus. Such genetic reassortment may be extremely important if properties such as transmissibility, virulence or the ability to infect a particular host are governed by one or more of the other genes.

Influenza infections in man

Despite the long history of disease recognizable as influenza in man, it is only in the virological era of the last 20–30 years that techniques have been available to enable effective epidemiological studies of the disease in humans (Kilbourne 1975, Pereira 1980). In the 20th century there have been three major pandemics interspersed with both minor and major epidemics. It appears that at frequent but irregular intervals between the major pandemics, which are caused by viruses antigenically 'new' to the host population (antigenic shift), variants of the pandemic viruses arise which are sufficiently different antigenically to be

capable of passing the immunological barriers of a proportion of the population and thus cause an epidemic. In general, each new 'epidemic' variant appears to wane gradually in its ability to find new susceptible hosts and dies out; it is then replaced by the next epidemic variant. These variants would appear to arise due to gradual change, i.e. by mutation and selection, of the original pandemic virus (antigenic drift) and this can be reproduced in the laboratory. Occasionally, the epidemics occurring between pandemics may be sufficiently severe and widespread to mimic a true pandemic. In 1946 an influenza A virus produced worldwide infections that were considered by some to represent a pandemic. However, epidemiological patterns were unlike those of true pandemics, and recent antigenic analysis has indicated that the virus was a variant of the H1N1 subtype rather than representative of an antigenic shift. The viruses prevalent between 1918 and 1957 (Table 2) were originally considered to be of three distinct subtypes – HswlN1, HON1 and H1N1 – but more recent analysis has indicated that these groupings really represent marked drift rather than antigenic shift, and all three are now placed in the H1N1 subtype (Schild *et al.* 1980). In past years introduction of pandemic virus has resulted in complete replacement of the previous virus, but the reoccurrence of H1N1 virus in 1977 did not result in the replacement of virus of H3N2 subtype and both viruses continue to circulate in the population. The influenza pandemic which arose in 1968 was caused by a virus of H3N2 subtype which showed a shift in haemagglutinin but not neuraminidase antigen. The ability of this virus to cause a severe pandemic is a reflection of the greater significance of antibodies to the haemagglutinin than to the neuraminidase for protection of the infected host.

The phenomenon of antigenic variation by shift and drift in influenza A viruses contrasts with influenza B viruses which show antigenic drift but not antigenic shift, resulting in regular epidemics but not explosive pandemics.

Influenza infections in other animals and birds

Although influenza may be used as a general term for respiratory illness in animals, as it is in humans, there are many historical reports of influenza occurring simultaneously or sequentially in man and domestic animals (Beveridge 1977). The term 'swine influenza' was applied to a 'new' disease of pigs seen during the 1918 pandemic in humans because of the close similarity of the clinical signs (Dorset *et al.* 1922). Some years passed before Shope isolated the causative virus of swine influenza in 1930 (Shope 1931), but this still preceded the first isolation of influenza virus from humans by three years (Smith *et al.* 1933). It was known that influenza virus of H1N1 (HswlN1) remained endemic in pig populations, particularly in the USA, but little consideration was given to this or to the possibility of influenza infections of other animals until 1955. In this year it was demonstrated that a virus which had been isolated and described as a filterable agent in 1901 (Centanni & Savonuzzi, cited by Stubbs

Table 2. Prevalence of influenza A subtypes in man

Period	Subtype
1918–1933	H1N1 (HswlN1)
1934–1946	H1N1 (H0N1)
1947–1957	H1N1 (H1N1)
1957–1968	H2N2 (H2N2)
1968–	H3N2 (H3N2)
1976	H1N1 (HswlN1)
1977–	H1N1 (H1N1)

Table 3. Evidence of interspecies transmission of human-associated virus of H3N2 subtype

Natural infection in:	Experimental infection in:
Swine	Swine
Cattle	Cattle
Chickens	Chickens
Turkeys	Dogs
Dogs	Cats
Cats	Ducks
Seabirds	Gibbons
Whales	Monkeys
Ducks	Baboons
	Rodents
	Ferrets

1965), and shown to be the causative agent of a disease of chickens known as 'fowl plague', was a type A influenza virus (Schafer 1955). In 1956 serological and virological evidence was obtained of influenza A viruses in horses (Heller *et al.* 1956, Sovinova *et al.* 1958) and in the next two years respiratory disease in horses caused by this virus was widespread in Europe. These findings aroused the interest of many scientists working on influenza in man, which was further concentrated by the H2N2 pandemic of 1957. Since that time the World Health Organization has endeavoured to encourage and coordinate work on the epidemiology of animal viruses, particularly in their relationship to human influenza (Kaplan 1980). However, it is only in the latter half of the last decade that the true picture of the vast reservoirs of influenza viruses that exist in animals, particularly birds, has been formed.

Influenza A viruses have been isolated from an enormous range of animal species, covering a complete spectrum from budgerigars to whales, apparently as a result of natural infection. In addition, a similar variety of animals has been shown to be susceptible to experimental infection. As an example, the natural and experimental animal hosts from which viruses of H3N2 subtype, similar to those from humans in the 1968 pandemic, have been isolated or shown to be infected are listed in Table 3. Such a wide range of natural or experimental hosts is not seen with all influenza viruses.

Although there is an enormous diversity of animal species that have been shown to be susceptible to influenza A virus infections, three groups of animals appear to be far more important in terms of numbers and the epidemic/endemic nature of the disease than other animals: these are pigs, horses and birds.

Influenza in pigs

Influenza A virus of H1N1 (Hsw1N1) subtype was first isolated from pigs in the USA in 1930 (Shope 1931) and shown to be the causative organism of a disease, similar to influenza in humans, that had been first seen in pigs during the 1918 pandemic. Antigenically-related viruses have persisted in the pig population of the USA since that time. Virus of this subtype has been reported as infecting pigs in many other countries, and these are listed in Table 4. In some countries H1N1 (Hsw1N1) virus has remained endemic in pigs, while in others – such as England and Czechoslovakia – viruses of this particular subtype are no longer demonstrable in the pig population. Some of the countries listed in Table 4 have probably imported infected

Table 4. Countries reporting influenza in swine

H1N1 (Hsw1N1)	
USA	Singapore
Japan	(Czechoslovakia)
Israel	Poland
Italy	Belgium
Hong Kong	USSR
People's Republic of China	(France)
Federal Republic of Germany	(England)
H1N1 (H1 – after 1977)	
England	
France	
Thailand	
H3N2	
Taiwan	France
England	Hong Kong
People's Republic of China	USA
Thailand	Japan
Federal Republic of Germany	Bulgaria

Table 5. Countries reporting equine influenza

H7N7 (Heq1Neq1) since 1956	H3N8 (Heq2Neq2) since 1963
Czechoslovakia	USA
Sweden	Canada
Germany	United Kingdom
USA	Switzerland
Canada	France
Poland	Brazil
Hungary	Mexico
Eire	Holland
France	Austria
United Kingdom	Hungary
Mexico	Italy
Yugoslavia	USSR
India	Lebanon
USSR	Greece
Austria	Japan
Iran	Yugoslavia
Argentina	Germany
Singapore	Iran
China	Finland
	Argentina

pigs from the USA, but the presence of virus of this subtype in pigs in the People's Republic of China, which has not traded with the USA, suggests that at least one other naturally-occurring virus pool exists. More recently, virus of H1N1 (Hsw1N1) has caused problems in pigs in European countries such as Belgium (Pensaert *et al.* 1981). This may be as a result of transmission from birds (see below).

Virus of H3N2 subtype was isolated from pigs in Taiwan in 1970 (Kundin 1970) and later demonstrated in the pigs of the countries listed in Table 4.

Influenza in horses

Two main subtypes of influenza A have been shown to affect horses: H7N7 (Heq1Neq1) first isolated in 1956 (Sovinova *et al.* 1958), and H3N8 (Heq2Neq2) first isolated in 1963 (Waddel *et al.* 1963). There have also been occasional isolated reports of natural infections of horses with H3N2, H2N2 and H1N1 subtypes (Tumova 1980). The two main subtypes may be regarded as having worldwide spread. Countries in which these viruses have been reported in horses are listed in Table 5.

Influenza in birds

Perhaps the most remarkable findings since the beginning of the era of animal influenza in 1955 has been the enormous number and variety of influenza viruses that have been shown to infect birds. All of the currently recognized 13H and 9N subtypes of influenza A have been isolated from birds, in most of the possible combinations (Hinshaw *et al.* 1981*b*). But it is the size of the virus pools that exist among feral, captive and domestic birds that is most notable.

The first isolation of an influenza virus from a wild bird was from common terns (*Sterna hirundo*) in 1961 (Becker 1966), but it has been only in the last decade that the true situation concerning the vast reservoirs of virus in wild birds has been fully realized. Earlier studies examined the respiratory tract of birds for the presence of virus and had only limited success in terms of virus isolation. But the observation by Slemons *et al.* (1974) that influenza viruses were readily isolated from cloacal swabs of naturally-infected waterfowl paved the way for further, more extensive studies.

Influenza A viruses have been shown to infect naturally birds from every major group of the class Aves (for reviews *see* Lvov 1978, Hinshaw *et al.* 1981*b*, Alexander 1982*a*, *b*). But perhaps the greatest carriers of influenza viruses are waterfowl, particularly ducks. Surveillance studies of these birds have resulted in the isolation of numerous subtype combinations in many countries throughout the world (Table 6). In some instances, extremely high isolation rates

Table 6. Examples of influenza A subtypes isolated from feral waterfowl in various countries since 1974

	Influenza A subtypes	
Country	Haemagglutinin	Neuraminidase
USA	H1, H3, H6, H7, H11	N1, N3, N6, N8, N9
Canada	All but one H	All N
France	H1, H3, H6	N2-N5, N8
USSR	H3, H10	N? N5
Egypt	H4, H11	N6
Japan	H1, H3, H5, H6, H7	N1-N3, N5 N7, N8
Federal Republic of Germany	H1-H4, H6, H11	N1-N3, N5, N6, N8, N9
Czechoslovakia	H3	N6
Hungary	H4-H6, H11, H?	N6, others not reported
Israel	H7	N2
Romania	H1	N1
England	H3, H6, H12	N2, N8

have been recorded. For example, Hinshaw *et al.* (1980*b*) reported positive isolations from 18–60% of the juvenile ducks and 4–27% of the adult ducks sampled on lakes in Alberta, Canada, during a three-year study in which nearly 5000 birds were swabbed. The importance of waterfowl is not only in the antigenic variety and size of the virus pools they harbour, but also the rapid dissemination of these viruses around the world due to the migratory nature of these birds.

Influenza infections of domestic poultry are extremely important to man in terms of the economic impact of the disease they may produce (Poss *et al.* 1982). Infection of such farm animals may also represent an interface between birds, humans and other animals. In this context it is extremely relevant that circumstantial evidence suggests that initial outbreaks in domestic poultry are nearly always the result of spread from wild birds (Easterday 1975, Alexander 1982*b*) and that, as a consequence, considerable antigenic variation is seen in disease outbreaks in domestic poultry (Table 7).

Captive, caged, pet birds may also have an important role to play in the propagation and dissemination of influenza viruses. Monitoring of such birds in many countries throughout the world has resulted in a high proportion of virus isolation (Alexander 1982*a*). An example of these studies was that conducted on captive birds dying in transit at Heathrow (London) Airport (Alexander *et al.* 1977). Between 1975 and 1978, virus isolation attempts from dead psittacines and passerines resulted in viruses being obtained from 20–25% of consignments examined. International trade of such birds inevitably involves air transport and it is worth noting that while the influenza-positive consignments had been shipped exclusively from India, they were bound for 14 different European airports.

It is important to appreciate the large amounts of virus produced by influenza A infections of bird populations. For example, the large numbers of ducks congregating on lakes in Canada prior to their migration south and the intestinal site of multiplication of influenza virus in these animals result in large doses of virus being excreted into the lake water (Webster *et al.* 1978, Hinshaw *et al.* 1979). Not only has it been shown that infections may be present at such levels to allow virus isolation from unconcentrated samples of lake water (Hinshaw *et al.* 1979), but that infectious virus may persist for over 30 days at 0°C and four days at 22°C (Webster *et al.* 1978). The presence and stability of influenza virus in such lake water offers a potential source of infection for birds and other animals.

Table 7. Summary of influenza A viruses from domestic poultry (chickens, turkeys and ducks)

	Influenza A subtypes		
Country	Haemagglutinin	Neuraminidase	Years in which reported
Scotland	H5, H?	N1, N7	1959, 1970
England	H1, H3-H7, H9-H11	N1-N4, N6-N9	1956, 1962, 1963, 1966, 1969, 1973, 1977, 1979–1982
Czechoslovakia	H4	N6	1956
Canada	H5, H6, H10	N1, N2, N7-N9	1952, 1963–1968, 1974
USSR	H3-H7, H11	N1, N2, N8, N9, N?	1960, 1963, 1967–1979
Australia	H7	N7	1975
Hong Kong	H1-H7, H9-H11	N1-N9	1969, 1975–1978
Italy	H5, H6, H10	N2, N8, N9, N?	1965, 1966, 1973, 1976–1980
Yugoslavia	H10	N?	1966
Poland	H3	N?	1967
Hungary	H?	N?	1970
Federal Republic of Germany	H2	N6	1972
USA	H1, H4-H7, H9	N1-N3, N5, N6, N8, N?	1964–1981
Belgium	H6, H11	N2, N6	1978, 1979
Israel	H5, H7, H11	N2	1971, 1973, 1978, 1980
France	H6, H9	N2	1979, 1980

H? and N?=subtype not identified

In many studies relatively small bird populations have been shown to be infected with more than one subtype of influenza A virus. That genetic reassortment may take place if two viruses infect a single bird has been well demonstrated (Webster *et al.* 1973, Webster & Campbell 1974, Hinshaw *et al.* 1980*a*) and two antigenically distinct influenza A viruses have frequently been isolated from a single sample from one bird (Shortridge *et al.* 1977*a*, Hannoun & Devaux 1980, Hinshaw *et al.* 1980*a*).

It may be concluded that enormous pools of both genetically and antigenically diverse influenza viruses exist within the bird population. The gregarious nature of many bird species, coupled with migratory and scavenging instincts, offer an indication of how genetic mixing may occur and how viruses may be freely transported around the world.

Emergence of pandemic viruses

The detection of vast pools of influenza viruses of many different subtypes among animals gave considerable impetus to research aimed at the major enigma of influenza in man – that is 'from where do the new subtypes which cause pandemics emerge?' The appearance of variant forms of influenza viruses that are responsible for epidemics between the major pandemics in humans is readily explained by the accumulation of point mutations and selection in the face of host immunity (antigenic drift). However, antigenic shift appears to be unlikely to be due solely to the occurrence of mutation and selection. The antigenic changes seen in shift occur too suddenly and, unlike antigenic drift, no intermediate viruses are detected; both H and N antigens may change when shift occurs, as in 1957; and, unlike drift, antigenic shift by selection cannot be mimicked in the laboratory. Work has, therefore, been channelled towards other possible explanations for the sudden appearance of pandemic influenza in human populations.

Hoyle & Wickramasinghe (1977) put forward the hypothesis that new viruses are formed in outer space and arrive on Earth in meteoritic dust. The re-emergence of H1N1 subtype in human populations and the apparent identity of this virus with viruses of pre-1957 origin led to suggestions of latency: Laver & Webster (1979) postulated that H1N1 viruses may have infected an organism, such as a lung parasite, which is capable of remaining dormant in man for many years. Such parasites do exist in humans in China where the recent H1N1 viruses first appeared. The idea of an intermediate host capable of transmitting virus was also put forward by Shope (1943) in his studies of influenza in pigs.

However, of the current theories the one most widely accepted is that by adaptation, probably involving genetic reassortment, transference of virus from other animals to man occurs which results in an antigenically novel virus with the ability to infect and spread in man. The reasons for a virus being more transmissible in one species than another are not clear, but are presumably genetically controlled by the virus. However, by genetic reassortment, or several reassortments, viruses may arise that possess the necessary genes to enable infection of man but may have surface antigens new to the host immune system.

Scholtissek *et al.* (1978) have produced evidence, based on RNA hybridization studies with isolated RNA segments, that viruses causing pandemics in 1957 and 1968 arose as a result of genetic reassortment. Their work indicates that 4 of the genes from H2N2 viruses which emerged in 1957 are identical with the corresponding genes in pre-1957 H1N1 viruses; while H3N2 virus, which emerged in 1968, had identity of all RNA segments, except for that coding for the haemagglutinin, with pre-1968 H2N2 virus. Interestingly, the segment coding for the haemagglutinin showed near identity with the HA segment in A/duck/Ukraine/1/63 H3(Hav7)N8). Scholtissek *et al.* (1978) concluded that these results indicated that H2N2 virus, responsible for the 1957 pandemic, arose as a result of genetic reassortment between pre-1957 H1N1 virus and an unknown virus, while H3N2 virus, responsible for the 1968 pandemic, arose as a result of genetic reassortment between pre-1968 H2N2 virus and A/duck/Ukraine/1/63(H3(Hav7)N8), or a very closely related virus, in which all but one gene (the haemagglutinin gene) was donated to the progeny virus by the H3N2 virus. Of course, the

new viruses may have arisen by reassortment or multiple reassortments of viruses unrelated to those already existing in man, but this would seem improbable.

The theory of adaptation/genetic reassortment allows that other factors may play a role in affecting the frequency of the emergence of pandemic virus. For example, the observation by Hope-Simpson (1978) that there is a direct correlation between sunspot activity and influenza pandemics was explained by Pereira (1980) as possibly due to the marked effect sunspot activity has on animal behaviour, particularly bird migration, which may result in greater mixing of both wild and domestic animals with the resulting transfer of viruses eventually on to man.

Evidence of transmission between man and animals

The theory that pandemic influenza viruses arise as a result of adaptation and/or genetic reassortment implies that viruses pass either from other animals to humans or vice versa, and that genetic reassortment then occurs by dual infection which results in progeny virus with the ability to infect and cause disease in humans, but with antigenic determinants different from recent viruses affecting the human population. Two animal viruses could reassort to produce a virus capable of transmission in man but this seems less probable. As stated above, there is some evidence that genetic reassortment occurred to produce the 1957 and 1968 pandemics in man (Scholtissek *et al.* 1978). This explanation of antigenic shift relies, at one stage or another, on the passage of virus across what in the past have been termed 'species barriers'. It would seem reasonable to suppose that such transference between species would occur many more times than when the conditions are optimal for the emergence of pandemic viruses. Studies in recent years have suggested that while viruses do not pass to and from man and other animals with complete freedom, under some conditions such transmission does occur.

Transmission between man and horses

Until the present century, and the development of the motor car, horses had a unique and intimate relationship with man; the opportunities for influenza viruses to pass to and from man and horses would thus have been far greater in the past than in present times. It is therefore of interest that in historical accounts of pandemics in man, frequent reference is made to similar disease in horses occurring either simultaneously or preceding that in man. Beveridge (1977) notes such references in the accounts of 12 pandemics occurring during the eighteenth and nineteenth centuries.

Serological studies have further revealed the presence of H3 (Heq2) antibodies in the sera of people born in the nineteenth century, and this has been considered as possible evidence that virus of this subtype was responsible for the pandemic of 1889–1890 (Tumova 1980). Experimental infection of human volunteers with H3 (Heq2) viruses has produced an influenza-like illness with virus-shedding and seroconversion (Kasel & Couch 1969, Cough *et al.* 1969). There is no evidence of infection of man with the other subtype of influenza, H7 (Heq1), which causes widespread epizootics in horses, and attempts at experimental infection have proved unsuccessful (Tumova 1980).

In addition to the evidence of spread of influenza virus from horses to man, there have been several isolated reports of infection of horses with subtypes H1N1, H2N2 and H3N2, usually associated with human infections (Tumova 1980). Experimental infection of horses with human-derived H3N2 virus has confirmed their susceptibility to this virus (Blaskovic *et al.* 1969).

Transmission between man and pigs

A disease similar to that seen in humans during the 1918 pandemic was also described in pigs in the USA at the same time. Further, this virus appears to have been maintained in the pig population of the USA and other countries since then. As influenza A virus was not isolated from pigs until 1930 (Shope 1931) or humans until 1933 (Smith *et al.* 1933), the exact relationship between the viruses in the human and pig populations during 1918–1930 cannot be determined. However, there is good serological evidence to suggest that the two viruses

were very closely related (Davenport *et al.* 1953). Similarly, theories suggesting the transmission of virus from pigs to humans resulting in the 1918 pandemic, or that pandemic virus passed from humans to pigs, are purely speculative. But there is evidence that the primary focus of the 1918 pandemic was among soldiers in Kansas, USA (Crosby 1976), and some epidemiologists were convinced that the 1918 virus was initially transmitted from pigs to man. This belief explains the extraordinary events occurring in the USA following what has become known as 'The Fort Dix Incident'. In January 1976 a virus of H1N1 (Hsw1N1) subtype, identical to viruses isolated from pigs in the USA, was isolated from a soldier who had died of influenza at Fort Dix, New Jersey, USA. At least five other servicemen were shown by virus isolation to be infected, and serological evidence suggested that some 500 personnel at Fort Dix were or had been infected with the same virus. With the 1918 pandemic in mind the US Government, in a unprecedented move, appropriated $100 million to produce sufficient vaccine for the entire population of the USA. The vaccination programme was started but eventually abandoned when it become clear that the virus had not spread any further.

The Fort Dix incident cannot be regarded as evidence of zoonosis, since pigs as the source of the virus, although likely, was never established. However, there is considerable evidence that transmission from pigs to man does often occur. Kluzka *et al.* (1961) reported that humans working with pigs in Czechoslovakia had antibodies to the H1 (Hsw1) subtype, and Schnurrenberger *et al.* (1970) reported that people in the USA who had close contact with pigs were more likely to have antibodies to H1 (Hsw1) than those that did not. Investigations in the USA during the mid 1970s gave further evidence of infections of humans with virus of H1 (Hsw1) subtype being related to contact with pigs (Smith *et al.* 1976, O'Brien *et al.* 1977, Easterday 1980). Final confirmation of the zoonotic nature of swine influenza came in 1976, when clinical influenza appeared in herd of pigs on a farm in Wisconsin two to three days before a caretaker also became ill with influenza. Viruses isolated from the pigs and the man were shown to be both antigenically and genetically identical H1N1 (Hsw1N1) influenza viruses (Easterday 1980, Hinshaw *et al.* 1978*a*). A further similar case was also seen, again in Wisconsin, involving a teenage boy who had been caring for pigs infected with influenza virus. In this case there was some evidence that the boy had transmitted the virus to two classmates (Easterday 1978).

As discussed above, the influenza virus, subtype H3N2, appears to be ubiquitous in animals and has on several occasions been isolated from pigs, or serological evidence of infections of this virus in pigs has been obtained (Kundin 1970, Harkness *et al.* 1972, Shortridge *et al.* 1977*b*, Styk *et al.* 1971, Chapman *et al.* 1978, Hinshaw *et al.* 1978*a*, Hannoun & Gourreau 1980, Shortridge & Webster 1979, Aymard *et al.* 1980, Nerome *et al.* 1981). In general, there is no evidence of pigs being infected with this subtype prior to the pandemic in humans in 1968. Indeed, the presence of a H3N2 subtype variant strain in the pig population of a country appears to coincide with the current epidemic strain infecting the human population (Aymard *et al.* 1980, Tillon *et al.* 1980, Nerome *et al.* 1981, Webster *et al.* 1977).

Further evidence of the spread of influenza viruses from humans to pigs was the appearance of H1N1 viruses (or antibodies to H1N1 viruses) – related to those circulating in the human population since 1977 – in pigs in France, England and Thailand after the appearance in the human population (Aymard *et al.* 1980, Hannoun & Gourreau 1980, Nerome *et al.* 1982, D H Roberts *et al.* unpublished).

Transmission between avian and mammalian species

There is no evidence of direct transmission of influenza virus from avian species to man. There is one report of the isolation of a virus of H7N1 (Hav1N1) subtype from a man who was suffering from hepatitis (Campbell *et al.* 1970), but no antibodies to that subtype were detected in the patient's serum.

Viruses antigenically identical to human variants of H3N2, H2N2 and H1N1 subtypes have been isolated from wild birds and domestic poultry (Bucher *et al.* 1980, Kovalchuk-Ivanyuk *et al.* 1975, Lvov 1978, Sazonov *et al.* 1977, Shortridge 1979, Shortridge *et al. 1979,* Hinshaw *et*

al. 1978*b*, Webster *et al.* 1975, Iftimovici *et al.* 1980). Several reports of disease in domestic fowl in the USSR have implicated strains of influenza A viruses normally seen only in human infections, and a temporal relationship to human epidemics has been suggested (Kovalchuk-Ivanyuk *et al.* 1975, Osidze *et al.* 1979). These reports refer to specific variants of influenza seen in human epidemics infecting birds. It should also be borne in mind that the original subtype Hav7 is now grouped with Heq2 and H3 in the H3 subtype, due to their close relationships (WHO Expert Committee 1980). Scholtissek *et al.* (1978) considered that the H3N2 virus responsible for the 1968 pandemic may have arisen as a result of recombination between H2N2 virus and a virus very closely related to A/duck/Ukraine/1/63 (H3(Hav7)N8).

Transmissibility of influenza viruses between birds and mammals may also be important in the origins of human influenza. Hinshaw *et al.* (1981*a*) showed that avian influenza isolates of several different subtypes were able to replicate in experimentally-infected pigs, ferrets and cats. One of the strains studied was transmitted from an infected pig to other pigs placed in contact. Mink (*Mustela vison*) have also been shown to be susceptible to experimental infection with avian influenza viruses, which may spread, by contact, to other mink (Matsuura *et al.* 1979, Yagyu *et al.* 1981).

Mohan *et al.* (1981) have reported the probable introduction of influenza virus to domestic turkeys by infected pigs on a farm in Ohio, USA. The pigs on the farm had shown an influenza-like disease after the introduction of new boars, with disease signs in the turkeys occurring immediately after the disease seen in pigs. Antibodies to H1 (Hsw1) subtype were detected in the sera of both turkeys and pigs.

Ottis & Bachmann (1980) reported the isolation of viruses of H1N1 (Hsw1N1) subtype from wild ducks in southern Germany which were antigenically similar to other H1N1 (Hsw1N1) isolates from wild ducks in North America (Hinshaw *et al.* 1978*b*). They further demonstrated, experimentally, the ability of the German isolates to replicate in pigs and to spread to other pigs placed in contact. There have been several other reports of H1N1 (Hsw1N1) viruses isolated from avian species in recent years (Alexander & Spackman 1981, Hannoun & Devaux 1980, Yamane *et al.* 1979, Pomeroy 1982, Butterfield *et al.* 1978). In 1979, outbreaks of influenza occurred in pigs in Belgium from which a virus of H1N1 (Hsw1N1) subtype was isolated (Pensaert *et al.* 1981). This virus was antigenically closely related to the viruses isolated from ducks in southern Germany and North America. Pensaert *et al.* (1981) considered this to represent strong supportive evidence of natural transmission of influenza A viruses from avian to mammalian species.

Another series of events of great significance in the epidemiology and ecology of influenza A viruses occurred in 1979–1980, beginning with the deaths of some 500 harbour seals (*Phoca vitulina*), about 20% of the population, around the Cape Cod Peninsula in North America. Deaths were diagnosed as due to acute haemorrhagic pneumonia, and an influenza virus of H7N7 (Hav1 Neq1) was repeatedly isolated from the lungs or brains of dead seals (Lang *et al.* 1981). Analysis of the virus isolated from seals both antigenically, using standard techniques and monoclonal antibodies, and genetically, using RNA hybridization techniques, indicated that the seal virus was most closely related to viruses from avian species (Webster *et al.* 1981*a*). During the initial studies, four people involved in post-mortem examinations of the seals had developed purulent conjunctivitis within two days of known contamination with seal material. Although no virological studies were done on these cases, in subsequent laboratory studies an infected seal, known to be shedding virus, sneezed directly into the eye of one of the investigators who developed conjunctivitis within two days. Virus identical to the seal virus was isolated from the affected eye for 4 days after this incident (Webster *et al.* 1981*b*). These incidents have been regarded as strong evidence that avian viruses may enter a mammalian population, causing a severe disease, and may then be passed on to man.

Conclusions

Explanations of the epidemiological trends of influenza A virus infections in man, which show minor epidemics occurring between pandemics, suggest that the minor epidemics occur

as a result of selection of variants arising from the preceding pandemic virus (antigenic drift), while pandemic viruses arise due to the sudden emergence of a virus with antigens new to all, or part, of the human population (antigenic shift). The most widely-accepted theory to explain the mechanism of antigenic shift is that of genetic reassortment between two viruses, resulting in a virus both transmissible in human and possessing novel antigens. There are enormous pools of influenza viruses in the animal population, especially birds, and in recent years studies on the ecology of these viruses have suggested that passage of viruses to and from humans and other animals occurs sufficiently regularly to allow dual infection and emergence of reassorted viruses. There is also good evidence to show that in some instances influenza may be a zoonosis. Virus has been shown to pass from infected pigs to man where it both causes disease and is transmissible.

The major developments in understanding the epidemiology and ecology of influenza in animals reviewed in this paper have occurred within the last 10 to 15 years. In this time there have been major advances in the understanding of the molecular biology of influenza viruses, which have governed the formulation of current concepts of the mechanisms involved in the epidemiology of influenza A virus in man (for review *see* Webster *et al.* 1982).

Great strides forward have been made in the understanding of 'the last great plague of man'. But much of the epidemiology, ecology and molecular biology of the influenza A viruses is still not understood. Webster *et al.* (1982) list over 30 questions pertinent to the understanding and eventual control of influenza. The recent events in the seal population of Cape Cod serve as a warning of how limited is our present knowledge. Should a virus capable of causing a similar disease in man enter the human population, it is doubtful that any of the advances made in the understanding of influenza viruses in recent years would do much to prevent a major pandemic similar to that seen in 1918.

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